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Is Universal Coverage Good for Neurons?

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In this issue of *Neuron*, Snider et al. analyze dendritic and axonal arbors of several cell types in several species. They show that general features of arbor structure are shared by the diverse cell populations, suggesting that the growth of these arbors is guided by universal principles.

The brain relies on precise connectivity to process information. It is hardly possible to specify each individual connection between the several billions of neurons in the average mammalian brain on the basis of a limited set of instructions contained in the genome. Therefore, it is often assumed that genes specify the general connectivity rules rather than specific connections. One such rule is that neurons that share similar properties project to proximal areas in the brain, leading to the emergence of brain maps. This allows wiring a large number of connections with only a few molecular markers that form gradients, such as in the case of topographic maps (McLaughlin and O'Leary, 2005). Another example is the columnar organization that is observed in many brain regions. This feature allows for the replication of similar connectivity modules several times throughout the area, thus limiting the number of variables needed to wire the circuit (Itzkovitz et al., 2008).

In this issue of *Neuron*, Snider and colleagues (Snider et al., 2010) propose a new simplifying principle that could dramatically reduce the number of variables defining the structure of axonal and dendritic arbors and, by extension, their connectivity. They suggest that both dendritic and axonal arbors can be described by a single density function. By definition, for a small volume of neuropil, the density function is proportional to the probability of finding a branch of a particular axon or dendrite in this volume. Snider et al. show that the density function for 3D mammalian arbors is close to a truncated Gaussian distribution. This is also true for two-dimensional goldfish and zebrafish arbors. Remarkably, they find that the density function is the same for different species (zebrafish, goldfish, mice, rats, cats, monkeys, humans), brain regions (cortex versus hippocampus), and cell types. The differences between arbors can be reduced to the variability in their linear dimensions and total lengths.

The authors demonstrate these results by first digitizing images of arbors using short line segments. Because the authors are interested in comparing arbor densities between different neurons, the exact locations of branches are not important. The idea is then to define a density function for each neuron as an average over individual branch positions. A direct calculation of the density function from branch segments will tend to be noisy due to their localized nature. One might attempt to reduce the noise by bundling arbors with certain properties. It is not clear, however, how to define these arbor properties from first principles. An alternative method is therefore needed to determine the arbor density profiles. The authors realized that by treating the density as a probability distribution, the entire arbor can be described in terms of global parameters called statistical moments. These moments include, for example, the mean and the variance of the density distribution, and higher moments. Moments can be calculated in a straightforward manner for each individual arbor. The first several moments (e.g., the mean, the variance, etc.) carry information about smoothened density profiles and are not sensitive to the detailed positions of individual branches. These moments allow for the comparison of branch densities between different cells, which makes them particularly useful in the search for common features shared by arbors. This observation is at the basis of the authors' method. Higher moments become more sensitive to the individual branch positions and, therefore, are not as useful for comparing different arbors.

Using this method, the authors address a variety of questions pertaining to the structure of axonal and dendritic arbors. The studies are based on a set of fish retinal ganglion cell arbors, both axonal and dendritic, that are approximately two-dimensional, and the three-dimensional reconstructions of cortical and hippocampal cells collected from a variety of mammalian species. In the cases where axons form disconnected arborizations in different areas, these arborizations are treated as separate entities. For pyramidal cells, the statistical moments are calculated for the apical and basal dendrites separately. The moments are calculated relative to the center of mass that is found for each arbor independently. The system of coordinates is then rotated for each cell so that the correlations between the three coordinates x, y, and z disappear.

Given the shapes of reconstructed arbors in shifted and rotated coordinates, the authors ask whether the arbor densities in each direction x, y, and z (x and y for fish arbors) are statistically independent. That is, if the arbor density is given by a function of three coordinates, can one assume that this distribution is a product of three one-dimensional distributions depending on each coordinate separately? A positive answer to this question would imply a substantial simplification of the arbor density profile: instead of a two- or three-dimensional function in which all three variables are statistically tangled, it would become a product of three separate one-dimensional functions. Therefore, this property of statistical independence is called separability by the authors.

Separability of arbor density can be illustrated as follows (Figures 1A and 1B). We illustrate this concept for a planar two-dimensional arbor; the three-dimensional case is similar. If one projects the density of an arbor onto one of the two coordinates, say x, one obtains the density function for this single coordinate, called the marginal distribution. The marginal distribution can be defined for the y coordinate as well by projecting the arbor density on the corresponding axis. For separable arbor densities, these two marginal distributions contain complete information about the arbor density (Figure 1A). This implies that one can represent the arbor density as a product of two (three in the three-dimensional case) marginal distributions. This is possible if one disregards the details of the arbor structure, such as the locations of individual branches. Alternatively, if the two marginal distributions do not contain complete information about the arbor density, it cannot be considered separable (Figure 1B).

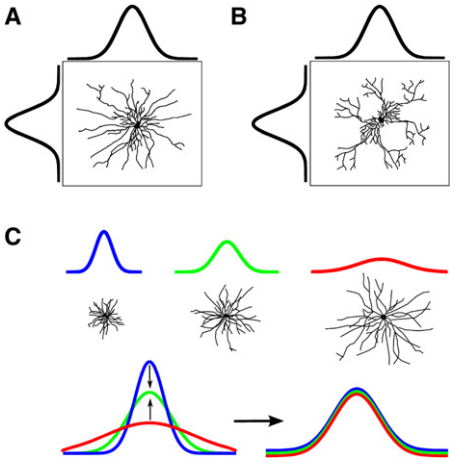

Figure 1. Separability and Self-Similarity of Arbors

(A) For separable arbors, marginal distributions (projections of arbor density on the two axes) carry complete information about smoothened branch density. In this case, both the marginal and smoothened two-dimensional densities are Gaussian.

(B) For nonseparable arbors, the marginal distributions do not carry complete information about branch density. Information about the chessboard-like structure in the two-dimensional density is lost given that the marginal distributions are simply Gaussian, as in (A).

(C) Self-similarity of arbor densities implies that density profiles of different cells can be morphed onto each other with a spatial transformation (contraction or expansion) coupled with a power-law rescaling.

By examining the moments of the distribution of arbor branches, Snider and colleagues conclude that both axonal and dendritic arbor densities can be considered separable with high precision. They find, for example, that moments of the distribution that involve different coordinates can be represented as products of one-dimensional moments—a property that is necessary for separability. The authors have assembled a large database of axonal and dendritic shapes coming from different species and cell types, allowing for the examination of cells that vary substantially in size over several orders of magnitude. This diversity of data admits the comparison of statistical moments over a large range of parameters, contributing to the robustness of the authors' findings.

Another property that can be defined for the arbors is self-similarity. Can arbor densities for neurons of different sizes be morphed into each other with a simple spatial contraction or expansion (Figure 1C)? If this were true, we could argue that the same master-plan is used to build arbors of different sizes and cell types across different species. Only a few parameters would be needed to define any particular arbor: dimensions, overall length, and orientation. By studying the moments of individual arbors, the authors find that they can emerge from a single shape template. The neuronal arbors of different sizes are therefore similar to one another, or, as mathematicians define it, self-similar. This claim pertains only to the smoothened density profiles and does not include the detailed branching pattern. It is surprising, however, that the branching rules that determine the overall density of processes in different species share some similarity.

The authors' analysis pertains to the properties averaged over arbors of a certain size. The moments and density function were in essence evaluated by averaging these parameters for many arbors of a given volume. It is this average density function that is found to be self-similar. The fluctuations in arbor shapes around the average were not considered. Numerically, the deviations of statistical moments from regression lines are not too large. It is quite possible, however, that individual arbors deviate from the "master-plan." Nonetheless, the similarity in the average density profiles among neurons of different origins and sizes is hard to predict. The magnitude and the nature of deviations from the average density profile are important to understand in future studies.

What is the density profile that arbors of diverse sizes and species conform to? The authors argue that it is the truncated Gaussian distribution. The truncation occurs at about two standard deviations for both the two- and three-dimensional case. This conclusion is fairly robust. For example, if the Gaussian distribution is truncated at three standard deviations from its peak, the observed moments are not consistent with the theoretical ones. The Gaussian distribution is noteworthy because it is the only function that can be rotationally symmetric and separable. Thus, the authors argue, nature has found a simple solution for the average arbor shape that satisfies both the constraint of separability and self-similarity.

Besides simplicity, this work addresses the principles of how connectivity is established in the brain. Are connections made locally in the real or functional space? The former possibility implies that neurons make synapses with other neurons based on their location. The latter option would mean that selectivity in connections is based on neuronal properties rather than on simple proximity. In many cases, when this question is asked explicitly, the connections are found to be organized based on functionality. In the ferret visual cortex, for example, connectivity is strongly dependent on the difference in the neuronal directional selectivity (Roerig and Kao, 1999). In layer four of the macaque monkey visual cortex, the shapes of dendritic arbors are biased by the presence of ocular dominance columns, implying the function-based selectivity of connections (Katz et al., 1989). In the olfactory bulb of rats, the inputs into mitral cells are organized in a spatially nonlocal manner (Fantana et al., 2008). Can these observations be reconciled with the local principle of the organization of neuronal arbors?

When describing connectivity one should distinguish between potential and real synapses. Indeed, locally organized arbor densities imply that neurons have a potential to be connected that depends on their relative position. An axon and a dendrite can make a synapse if their branches happen to pass near each other. Such axonal and dendritic encounters are called potential synapses (Stepanyants and Chklovskii, 2005). The number of potential synapses between two cells depends on the overlap of their arbor densities, i.e., exactly the quantity studied by Snider et al. According to Snider and colleagues, this overlap depends on the relative position of two cells. Thus, these findings suggest a local organization of potential connectivity. On the other hand, the decision to promote a potential synapse to a real one may depend on the properties of cells, such as cell type or receptive fields. The principle for organization of connections that emerges from this study is this: the potential connectivity depends on proximity while the real connections may respect cells' functions. This difference could have an inherent utility: if potential connections are indiscriminate, it is easier to rewire the real connections later if necessary (Holtmaat and Svoboda, 2009; Wen et al., 2009).

This theory suggests an arbor-centric view on how connections are made in the brain. First, an arbor is formed that provides potential synapses. Then the potential synapses are converted to actual ones based on functionality or cell identity. An alternative viewpoint is emerging from the studies of axonal and dendritic branching in the retinotectal system. In the zebrafish for example (one of the species studies by the authors) as well as in *Xenopus*, one can observe how branches and synapses are formed online, using time-lapse imaging. These studies show that synapses and branches are formed simultaneously though the process of trial and error (Hua and Smith, 2004). Moreover, from monitoring the dynamics of synapse and branch formation, support emerges for the "synaptotropic hypothesis," i.e., the synapse-centric view on branch formation. According to this hypothesis, synapses could guide the growth of arbors by stimulating the formation of new branches or by stabilizing the existing ones (Hua and Smith, 2004). Thus, new branches are preferentially formed near synapses (Alsina et al., 2001; Javaherian and Cline, 2005), with the likelihood of nascent branch initiation locally regulated by the individual synapses (Meyer and Smith, 2006). This regulation may be influenced by correlations in neural activity (Tsigankov and Koulakov, 2009). It is therefore not obvious how universal arbor structure can emerge in the presence of growth rules that are both local and activity/identity dependent.

One possibility is that cortical connectivity is formed by rules different from the ones in the tectum. However, Snider and colleagues did include retinotectal axons into consideration. Another option is that axonal and dendritic branches are affected by local synapse-based regulation, yet the global structure that is described by the density of arbors is universal. Therefore, when a large number of independent random variables is added, the sum has the universal Gaussian distribution. Finally, it is possible that the arbor density function of universal shape emerges as a result of some common guidance mechanism that specifically controls the density profile. An example of such a mechanism is the enforcement of retinal mosaics by DSCAMs, which ensures the complete coverage of visual space by the dendrites of retinal ganglion cells (Fuerst et al., 2009). In the absence of DSCAMs, neurons in the mouse brain clamp together (Fuerst et al., 2009), which leads to the possibility that complete coverage may exist beyond the retina, i.e., is universal. These possibilities will be interesting to disambiguate in future studies.

In this issue of *Neuron*, Snider et al. make a strong case for universality in the geometry of axonal and dendritic arbors. The smoothened arbor densities are found to be similar for a diverse set of neurons ranging in size, cell type, brain region, and species. It is possible that only a few parameters are needed to define the geometry of individual arbors. Although the mechanisms for the emergence of universal arbor shapes are poorly understood, these findings suggest a potent simplifying principle for the organization of brain connectivity.

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